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RESEARCH ARTICLE

Prevalence of intestinal parasites in leafy green vegetables consumed by inhabitants of Jeddah city, Saudi Arabia

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ABSTRACT

Vegetables are a crucial component of every diet and a good source of vitamins and minerals. Many raw vegetables, such as salads, are consumed, and consuming raw vegetables contaminated with human and animal feces can lead to a variety of intestinal parasite diseases. The goal of this cross-sectional study was to investigate the incidence of contamination of green leafy vegetables with human intestinal parasites, as well as identify the most common parasite species and any seasonal variation in parasite abundance in green leafy vegetables found and sold for human consumption in various markets and stores in Jeddah. To the best of our knowledge, no research has been conducted on the prevalence of parasite-contaminated vegetables in Jeddah. From September 2020 to July 2021, 250 leafy vegetables (coriander, watercress, lettuce, parsley, leek, and green onion) were collected from various markets and stores in Jeddah. The vegetables were sorted, labelled, and evaluated in the laboratory after being completely washed with distilled water, examined for sediment, staining, and microscopically examined. Chromatographic immunoassay tests were also used to confirm parasite identification. Intestinal parasites were discovered in 35.2% of the vegetables (88/250). Coriander was the most contaminated (64.2%), while green onion was the least contaminated (21.9%). *Blastocystis spp.* was the most often discovered parasite (55.7%), followed by *Strongyloides spp.* larva (22.7%) and *Entamoeba coli* cysts (6.8%). Summer has the greatest percentage of parasites, followed by autumn, while winter has the fewest. There was no statistically significant relationship between the kind of vegetable, seasonal parasite prevalence in green vegetables, and parasite type.These findings highlight the importance of inspecting vegetables grown or imported from endemic countries for parasite contamination. In Jeddah, several fresh green vegetables were tainted with intestinal parasites. This demonstrates that those who consume raw vegetables regularly may be susceptible to parasitic illnesses. These findings highlight the need of monitoring vegetables grown or imported from parasitic-endemic areas. As a result, it is critical to establish strategies and control measures to limit the likelihood of parasites in food-borne diseases.

Keywords: contamination; intestinal parasites; Jeddah; leafy vegetables

INTRODUCTION

Consumption of fruit and vegetables is important for a healthy lifestyle. Vegetables are a needed part of an individual's meal and a great source of vitamins and essential minerals. Many types of vegetables are eaten raw, such as salads. This may increase the risk of acquiring numerous pathogens such as viruses, bacterial and parasitic organisms.

The prevalence of these infections has considerable implications for public health and is projected to impact approximately 3.5 billion individuals worldwide (Hotez et al., 2009). Many countries, including Egypt, Libya, Iran, Iraq, and the Philippines, have been the subject of multiple studies examining the potential risk of parasite transmission via contaminated raw vegetables (Abougrain et al., 2010; Gharavi et al., 2002; Hadi, 2011; Hassan et al., 2012; Kpoda et al., 2022; Said, 2012; Shahnazi & Jafari-Sabet, 2010; Slifko et al., 2000; Su et al., 2012). The ingestion of raw vegetables without appropriate washing is a vital source of parasitic disease transmission (Beuchat, 2002). Also, sewage water has been used for irrigation in many

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developing countries along with human and animal excreta as fertilizers (Orlandi et al., 2002). This increased the spread of parasites in many non-endemic regions in different countries (Geary & Haque, 2021). In addition, they import fruits and vegetables from various countries with unknown hygiene practices in their processing (Gemechu et al., 2023). Taking into account that a quantity of the vegetables grown in these developing countries is exported to various countries, the risk of dissemination of these contaminations to other countries cannot be ignored (Gupta et al., 2010).

Unhygienic practices during production, transport, processing and preparation by handlers can also contribute to vegetable contamination (Okyay et al., 2004).

Intestinal parasitic infections continue to be a health concern worldwide. Furthermore, they can also impair the physical and mental growth of children(Kozan et al., 2005; Stephenson et al., 2000; Stoltzfus et al., 2004).In the past decade, incidences of human parasitic diseases have also been attributed to the consumption of raw fruits and vegetables (Drake et al., 2000). It is estimated that approximately one billion individuals are infected with intestinal helminths such as *Ascaris lumbricoides*, *Trichura trichiura* (795 million), and hookworms (740 million) worldwide (Guyatt, 2000). Inadequate sanitation, unclean water, and substandard sanitary conditions may be playing an important role by contributing to infections with medically significant parasites including intestinal worms and protozoa (De Silva et al., 2003; Wegayehu et al., 2013). In addition, substandard agricultural practices during production, transport, processing, and preparation by handlers could also contribute to produce contamination.

To the best of our knowledge, there is a lack of published research concerning the level of parasitological contamination of fruits and vegetables in Jeddah, Saudi Arabia. Thus, the objectives of the current study is aimed to determine the factors associated with parasitic contamination of selected vegetables commonly consumed and the factors associated with their spread. Moreover, to investigate parasitic species' prevalence variation according to the area, season, and hygiene applications.

MATERIALS AND METHODS

Study area

A cross-sectional study was conducted in Jeddah, located on the Red Sea coast in the Western region of Saudi Arabia (latitude 29.21 North & longitude 39.7 East). It is Saudi Arabia's second-largest city after the capital, Riyadh, and the largest city in Makkah Province.

Sample collection and preparation

Fresh leafy green vegetable samples were randomly collected from different areas in Jeddah from various commercial groceries and markets during different seasons from September 2020 to July 2021. Before being placed on the shelves of marketplaces, these vegetables were typically pre-washed, and a total of 250 fresh vegetable samples were collected.

Six types of leafy green vegetables that are commonly consumed raw by individuals were chosen. The samples of fresh vegetables collected for this study were lettuce (*Lactuca sativa*), parsley (*Petroselinum hortense*), watercress (*Eruca sativa*), coriander (*Coriandrum sativum*), green onion (*Nasturtium officinale*) and leek (*Allium porrum*).

The leafy green vegetables were placed in sterile, labelled plastic bags before being transported to the Laboratory of Medical Microbiology and Parasitology at the Faculty of Medicine, King Abdulaziz University for parasitic examination. Each fresh vegetable sample was prepared according to Al-Megrin (Al-Megrin, 2010) and Abougrain, et al. 2010 (Abougrain et al., 2010). To eliminate large debris, the solution was filtered through a sieve with a pore size of 425 µm and transferred to 50ml tubes, and centrifuged at 3000rpm for 10 minutes. Following that, the supernatant was pipetted and discarded, and the sediment was carefully collected and examined using a light microscope with 10x and 40x objectives. To increase the likelihood of parasite discovery, three slides were made from each sample. An iodine-stained smear was also made, and morphological features were used to identify the eggs, larvae, and parasite cysts as previously described (Soulsby, 1968). In addition, Modified Zeihl–Neelsen stain was used to identify *Cryptosporidium* spp. protozoal oocysts (Fayer et al., 2000).

Statistical analysis

SPSS 20.0 was used to analyze the data. The Chi-square test was used to determine the relationship between the type of parasite and the discovery of parasitic infection in fresh vegetables, as well as the significance of the type of contaminated parasite and the kind of vegetable and season. The p value<0.05, was considered significant.

RESULTS

A total of 250 fresh leafy green vegetable samples were examined and analyzed for the presence of intestinal parasite contamination. Helminthic eggs, larvae and protozoan cysts were detected in 35.2% (88/250) of the fresh vegetables examined (Table 1).

The vegetable with the highest contamination with parasites was the coriander (64.2%), followed by watercress

(40.4%), leek (30.7%), lettuce (27.9%), parsley (25.5%), and green onion (21.9%) as shown in Table 2. Interestingly, the highest detected parasites in the leafy green vegetable samples were *Blastocystis hominis* (55.7%), *Strongyloides spp.* larva (22.7%), *Enatmoeba coli* (6.8%), *Hymenolepis spp.* egg (5.7%), hookworm eggs (2.3%), *Endolimax nana* cyst (2.3%), *Taenia spp.* egg, *Toxocara spp.* egg, and *Ascaris lumbricoides* egg (1.1%) for each (Table 2).

Direct wet smear detection and modified Zeihl-Neelsen staining did not detect *Cryptosporidium* species, or *Entamoeba dispar/ histolytica* in the samples. The season with the highest number of parasites was summer followed by autumn and the least was winter. Samples were not collected in spring (Table 3).

The correlation between the type of vegetable and parasite was not statistically significant (chi-square p-value<0.582).

Table 1: Prevalence rate of parasitic contamination on fresh leafy vegetables

Parasites	n	%
Ascaris lumbricoides egg	1	1.1
Blastocystis spp.	49	55.7
Entamoeba coli	6	6.8
Larva of *Strongyloides spp.*	20	22.7
Hymenolepis spp.	5	5.7
Toxocara spp. egg	1	1.1
Taenia spp. egg	1	1.1
Endolimax nana cyst	3	3.4
Hookworm egg	2	2.3
Total	88	100.0

Data presented as numbers and percentages.

In addition, there was no statistically significant relationship between the type of parasite and season prevalence in leafy green vegetables (p-value < 0.545).

DISCUSSION

Vegetables are an essential component of a healthy diet, and many of them are consumed raw. Thus, they may play a crucial role in the transmission of parasitic pathogens spread by food (Geary & Haque, 2021; Kpoda et al., 2022; Nazemi et al., 2012). Examining vegetables sold in markets and stores for parasite contamination could highlight improper vegetable harvesting and handling practices and highlight the prevalence of intestinal illnesses among local entities (Alhabbal, 2015). The recovery of parasites from vegetables enriched our perception of the possible source of pathogenic parasite acquisition in this study area. Vegetables are highly consumed daily by residents of Jeddah city, and the vegetables chosen in this investigation were chosen according to the commonest consumed vegetables, usually eaten raw in prepared salads. This study revealed that six types of fresh leafy green vegetables commonly consumed by Jeddah city inhabitants were contaminated with many intestinal parasites. Several previous studies on the parasitic contamination of vegetables have been conducted in neighboring countries and other cities in Saudi Arabia. However, to our knowledge, no studies have been reported in Jeddah, Saudi Arabia's second-largest city.

In the present study, 250 samples of six different leafy green vegetables were examined from various wholesale, retail,

Table 2: Distribution of parasitic contamination of leafy green leafy vegetables

Parasites	Vegetables						
	lettuce	parsley	watercress	coriander	green onion	leek	Total
Ascaris lumbricoides egg	1	0	0	0	0	0	1
	8.3%	0.0%	0.0%	0.0%	0.0%	0.0%	1.1%
Blastocystis spp.	9	6	7	18	3	6	49
	75.0%	54.5%	41.2%	66.7%	33.3%	50.0%	55.7%
Entamoeba coli	0	2	1	1	0	2	6
	0.0%	18.2%	5.9%	3.7%	0.0%	16.7%	6.8%
Larva of *Strongyloides spp.*	2	2	5	5	4	2	20
	16.7%	18.2%	29.4%	18.5%	44.4%	16.7%	22.7%
Hymenolepis spp. egg	0	0	2	1	1	1	5
	0.0%	0.0%	11.8%	3.7%	11.1%	8.3%	5.7%
Toxocara spp. egg	0	0	0	1	0	0	1
	0.0%	0.0%	0.0%	3.7%	0.0%	0.0%	1.1%
Taenia spp. egg	0	0	0	0	0	1	1
	0.0%	0.0%	0.0%	0.0%	0.0%	8.3%	1.1%
Endolimax nana cyst	0	1	1	1	0	0	3
	0.0%	9.1%	5.9%	3.7%	0.0%	0.0%	3.4%
Hookworm egg	0	0	1	0	1	0	2
	0.0%	0.0%	5.9%	0.0%	11.1%	0.0%	2.3%
Total	12	11	17	27	9	12	88
	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

Data presented as numbers and percentages.

Table 3: Seasonal prevalence of intestinal parasites in leafy green vegetables in Jeddah

Parasites	Season			
	autumn	winter	summer	Total
Ascaris lumbricoides egg	1	0	0	1
	2.7%	0.0%	0.0%	1.1%
Blastocystis hominis	18	7	24	49
	48.6%	58.3%	61.5%	55.7%
Entamoeba coli	3	0	3	6
	8.1%	0.0%	7.7%	6.8%
Larva of *Strongyloides spp.*	9	4	7	20
	24.3%	33.3%	17.9%	22.7%
Hymenolepis spp. egg	4	0	1	5
	10.8%	0.0%	2.6%	5.7%
Toxocara spp. egg	1	0	0	1
	2.7%	0.0%	0.0%	1.1%
Taenia spp. egg	1	0	0	1
	2.7%	0.0%	0.0%	1.1%
Endolimax nana cyst	0	1	2	3
	0.0%	8.3%	5.1%	3.4%
Hookworm egg	0	0	2	2
	0.0%	0.0%	5.1%	2.3%
Total	37	12	39	88
	100.0%	100.0%	100.0%	100.0%

Data presented as numbers and percentages.

and supermarket markets in Jeddah. In addition, *Blastocystis spp.*, *Strongyloides l*arva, *Entamoeba coli* cysts, *Endolimax nana* cysts, hookworm eggs, *Ascaris lumbricoides* eggs, *Hymenolepis spp.* eggs, *Toxocara spp.* eggs, and *Taenia spp.* eggs were found in the examined vegetables.

This investigation revealed that the prevalence of parasite infection in vegetable samples from Jeddah City, in the western region of Saudi Arabia, was 35.2%, which was comparable to recent results from Thailand (Punsawad et al., 2019), Iran (Fallah et al., 2016), and Syria (Al Nahhas & Aboualchamat, 2020; Alhabbal, 2015), Pakistan (Maqbool et al., 2014), and Poland (Klapec & Borecka, 2012), where the prevalence ranged from 31.23%–35.1%, respectively.

Previous research revealed greater levels of vegetable contamination in Casablanca, Morocco (62.5%) (Hajjami et al., 2013), Tripoli, Libya (58%) (Abougrain et al., 2010), Iran (44.8%) (Ebrahimzadeh et al., 2013; Olyaei & Hajivandi, 2013), and Nigeria (40%) (Amaechi et al., 2016; Ogbolu et al., 2009). Iraq accounted for 51% and 48.8% (Mirzaei et al., 2021; Mohammed et al., 2019).

In accordance with our findings, investigations conducted in Tabuk City, Saudi Arabia, revealed a greater prevalence of infected vegetables with parasites (46%) (Gabre & Shakir, 2016). On the other hand, studies in Riyadh (Al-Megrin, 2010) and the United Arab Emirates (15.1%) (El Bakri et al., 2020) revealed lower percentages of contamination in vegetables. Coriander had the highest parasitic contamination (64.2%). The current study was in acquiescence with many extensive studies; in Brazil (Rodrigues et al., 2020), Thailand discovered 44.8% contamination in coriander (Punsawad et al., 2019), and in Khyber Pakhtunkhwa, Pakistan (14.2%) (Khan et al., 2017) in that coriander had the highest prevalence of contamination.

In contrast, other studies have found the highest parasitic contamination in lettuce in Saudi Arabia (Al-Megrin, 2010), Egypt (Eraky et al., 2014; Said, 2012), Khartoum, Sudan, 36.4 % (Mohamed et al., 2016), Iraq, and Brazil (Luz et al., 2017).

According to our data, green onion had the lowest contamination rate (21.9%). This result was consistent with investigations carried out by Said et al. in Khorramabad, Iran (34.5%) (Ezatpour et al., 2013), Benha, Egypt (16.5%), and Alexandria, Egypt (13.3%) (Said, 2012), all of which were in Egypt. While the Western South Province of Saudi Arabia was found to have the highest vegetable contamination rates (28%) (Al-Binali et al., 2006). The epidemiology of the transmission of parasite pathogens is influenced by the type and number of investigated samples, the geographical location, irrigation methods, and post-harvest management practices of these vegetables (Said, 2012).

Blastocystis spp. was found to be the most prevalent intestinal parasite in the vegetables examined in the current investigation (55.7%). Patients who exhibit a variety of clinical symptoms (Mohamed et al., 2017; Stensvold et al., 2009).

In a study conducted, *Blastocystis sp.* was determined to be the second most prevalent parasite in vegetable samples (29.5%). Al Nahhas and Aboualchamat estimate 2020. Additionally, research conducted in Iran by Isazadeh et al. in 2020 found just 8.2% of vegetables to be contaminated with *Blastocystis sp.* (Isazadeh et al., 2020), while investigations conducted in Northern Brazil found 15% and in Riyadh found only 2.8% (Al-Megrin, 2010; Rodrigues et al., 2020).

According to numerous epidemiological investigations conducted in various countries (Tan, 2008), *Blastocystis spp.* is the most prevalent eukaryotic parasite detected in human feces. As seen in France (The ANOFEL Cryptosporidium National Network, 2010) and the United States (Boorom et al., 2008), the frequency of *Blastocystis spp.* is greater than that of other intestinal protozoan parasites such as *Giardia lamblia, Entamoeba histolytica, and Cryptosporidium spp.*

Strongyloides spp. ranked as the second most frequent source of contamination in this investigation, being discovered in 22.7% of the studied raw produce. Our results were in line

with earlier research from Jimma Town, Ethiopia, which found a prevalence of *Strongyloides spp.* contamination of 43% (Duedu et al., 2014), and in Thailand, found to be the second highest parasite identified (10.6%) (Punsawad et al., 2019). *Strongyloides spp.* possess a complex life cycle, which includes a stage of free-living in the environment without needing a host for reproduction; this may explain the high prevalence of contamination. Various regions have reported incongruent contamination rates with this larval form in vegetables comparable to those examined in this investigation.

The third most common parasitic contaminant (6.8%) was a non-virulent commensal *E. coli* cyst. This was detected higher in the UAE (18.2%), Khartoum city in Sudan (14.3%) and in the South-Western area of Saudi Arabia (19.04%) (Al-Binali et al., 2006; El Bakri et al., 2020; Mohamed et al., 2016).

Hymenolepis spp. eggs were found in 5.7% of the contaminated vegetables. *Hymenolepis* eggs were found in 3.0% of the vegetables analyzed in the UAE, with lettuce being the most commonly contaminated vegetable. The ova were found in similar numbers and vegetable types (lettuce) in Alexandria, Egypt (2.6%), and Mazandaran province, Iran (2.2%) (El Bakri et al., 2020; Rostami et al., 2016; Said, 2012).

Another commensal, *Endolimax nana*, was discovered in 3.4% of vegetable products. In the UAE, it was detected with a prevalence of 6.1% (El Bakri et al., 2020) and with comparable results It is significant to note that the presence of both of these protozoa is a sign that vegetables have been contaminated with human faeces.

The lowest detected parasites were the eggs of the helminth worms, hookworm (2.3%), *Ascaris lumricoides*, *Taenia spp.*, and *Toxocara spp.* (1.1%). On the contrary, other studies detected a higher percentage of contamination in Riyadh and Tabuk, Saudi Arabia (Al-Megrin, 2010; Gabre & Shakir, 2016).

Summer had the highest percentage of parasites, followed by autumn, and winter had the fewest. According to earlier studies, the rate of vegetable contamination was higher during warm seasons than it was during cold seasons, which was consistent with the effect of season on the prevalence of parasites (Daryani et al., 2008; Rostami et al., 2016). This finding reveals that climate and temperature are related to the transmission and prevalence of parasites. Statistically, however, there was no correlation between parasite prevalence and season type.

Despite statistical analysis not showing a significant correlation between the vegetable type and parasite occurrence ($p > 0.050$) or reliance on parasite incidence with the vegetable type ($p > 0.050$), our investigation demonstrates the potential of fresh raw produce to serve as a source of infection with a variety of parasitic pathogens. Vegetables like coriander, lettuce, spring onions, parsley, and watercress have been the subject of previous studies.

Another key conclusion is the need for proper cleaning and handling of vegetables. The unrestricted use of natural manure containing human and animal waste, as well as the use of sewage water for irrigation, are some of the practices prevalent in these nations (El Bakri et al., 2020; Eraky et al., 2014; Ogbolu et al., 2009).

It is not surprising that the prevalence rates of the various intestinal pathogens from fresh vegetables reported in the current study differ from those reported by others. Geographical location, the kind and quantity of samples tested, techniques used to identify intestinal parasites, and factors including the type of irrigation water utilized and post-harvest handling procedures, may be to blame for these discrepancies. The incidence of intestinal parasite contamination seen in the majority of the examined vegetables may be caused by several factors. Among these are the use of fresh poultry faeces as fertilizer and the watering of crops with dirty water. Market-related handling is another cause of contamination, particularly in areas without adequate hygienic provisions.

One of the study's limitations was the lack of vegetable sample collection in the spring. Furthermore, a greater number of vegetable collections are recommended, as is the use of molecular parasite detection. In addition, we propose future water and soil collection from farmers' local vegetables.

CONCLUSION

In conclusion, the presence of intestinal parasites in some fresh leafy green vegetables in Jeddah indicates a potential risk for individuals who consume raw vegetables on a regular basis. The incidence of food-borne parasitic infections is concerning and underscores the need to implement effective control techniques aimed at reducing disease transmission. These interventions may include improved sanitation practices during cultivation, harvesting and distribution of produce, as well as targeted education campaigns focused on safe food handling practices among consumers. Such measures are strongly advocated to mitigate risks associated with parasitic infections linked to consumption of contaminated fruits and vegetables in this region. Expanding academic research in this area could also yield insights into novel strategies that can further reduce

these risks while ensuring availability of nutritious produce options for all consumers across diverse demographics and socioeconomic backgrounds within Jeddah communities.

DISCLOSURE

The authors declared no conflict of interest.

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Conflict of interest

The authors declare they have no conflict of interest.

Authors contributions

Sarah A. Altwaim: Main principal investigator. (i) substantial contribution to conceptualization, design, analysis and interpretation of data; (ii) drafting & critical revision of important scientific content; (iii) approval of the version to be published.

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